

Chemical and natural stressors combined:

from cryptic effects to population extinction

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population extinctionAndré Gergs^{1,2}, Armin Zenker³, Volker Grimm^{4,5} & Thomas G. Preuss²¹Department of Environmental, Social and Spatial Change, Roskilde University, Universitetsvej 1, 4000 Roskilde, Denmark,²Institute for Environmental Research, RWTH Aachen University, Worringer Weg 1, 52074 Aachen, Germany, ³School of Life Sciences, Institute for Ecopreneurship, University of Applied Sciences and Arts Northwestern Switzerland, Grödenstrasse 40, 4132 Muttenz, Switzerland, ⁴Department of Ecological Modelling, UFZ Centre For Environmental Research Leipzig-Halle, Permoserstrasse 15, 04318 Leipzig, Germany, ⁵Institute for Biochemistry and Biology, University of Potsdam, Maulbeerallee 2, 14469 Potsdam, Germany.

In addition to natural stressors, populations are increasingly exposed to chemical pollutants released into the environment. We experimentally demonstrate the loss of resilience for *Daphnia magna* populations that are exposed to a combination of natural and chemical stressors even though effects on population size of a single stressor were cryptic, i.e. hard to detect statistically. Data on *Daphnia* population demography and along with model-based exploration of our predator-prey system revealed that direct trophic interactions changed the population size-structure and thereby increased population vulnerability to the toxicant which acts in a size selective manner. Moreover, population vulnerability to the toxicant increases with predator size and predation intensity whereas indirect trait-mediated interactions via predator kairomones may buffer chemical effects to a certain extent. Our study demonstrates that population size can be a poor endpoint for risk assessments of chemicals and that ignoring disturbance interactions can lead to severe underestimation of extinction risk.

Risk assessment of chemicals is strongly regulated in Europe and North America. For pesticides and other toxicants, risk is quantified via the ratio of a predicted environmental exposure concentration and a concentration causing a toxic effect (Europe) or its inverse (North America). Lethal and sublethal toxic effects, however, are determined in standard laboratory tests, with individual organisms. What these individual-level effects mean at the level of populations, communities, and ecosystems remains an open question. To take this uncertainty into account, safety factors are used in the estimate of concentrations that unlikely result in environmental effects, but their choice is arbitrary¹. So far, safety factors seemed to be conservative enough to prevent Rachel Carson's Silent Spring² but concern about the lack of ecological realism in current risk assessment schemes is increasing, since the number and intensity of stressors on natural populations have reached unprecedented levels. In particular, it remains unclear which endpoints or metrics for ecological risk assessment should be considered at the population level. Average abundance, intrinsic rate of increase, or recovery time have been proposed but do they really relate to risk in an ecologically meaningful way?

In ecology, focus has shifted over the last two decades from equilibria to negative feedback, transient dynamics³ and resilience, defined as the ability to maintain internal relationships and functioning despite disturbances and stress⁴. Resilience is the result of buffer mechanisms, which only have a certain capacity⁵. Stress beyond this capacity leads to sudden changes or regime shifts, which for populations may correspond to extinction. Regime shifts have been observed for ecosystems such as savannas and coral reefs but might also be an issue in species conservation, the most prominent example being the decline in honeybees and amphibians^{6,7}. So far, no satisfactory monocausal explanation of their decline has been found. Rather, multiple stressors might have caused the loss of their resilience.

For chemical stress, several studies have demonstrated that a combination of toxicants and species interactions can impose much higher risk on populations than the toxicant alone. Community processes that can alter the magnitude of toxicant effects include interspecific competition⁸, parasitism^{9,10}, presence of predator kairomones^{11,12} and predation^{13,14}. These processes affect not only abundance but also intraspecific competition and population size-structure. Since individuals' susceptibility might differ across life stages, body sizes, population



size-structure and the timing of exposure, there will be consequences for the susceptibility of populations to toxicants^{15,16}.

Here we raised the question of whether using population abundance as an endpoint for ecological risk assessment is sufficient to indicate the risk of losing resilience when populations are exposed to a combination of chemical stress and predator effects. As a model system we used the water flea *Daphnia magna*, a standard test species for ecotoxicology. It has previously been shown for the case of *Daphnia* that predator conditioned culture medium or the presence of predators altered egg size¹⁷, resulted in a higher age at maturity¹⁸ and in smaller clutch sizes¹¹. However, rapid juvenile development and high reproductive output were also observed¹⁹. Previous laboratory studies applying artificial predation by manually removing fixed portions of prey populations revealed alterations in prey abundance¹⁴ and species composition²⁰ as well as changes in recovery times after insecticide pulse exposure²¹.

Predation may interact with toxicant effects via size-selectivity of both stressors. Predators tend to specialize in particular sizes or developmental stages in their choice of prey types^{22,23}. Such size selectivity was frequently found in fish^{24,25} and invertebrate predators^{26,27,28}. The outcome of predation has also been shown to vary with predator size^{29–31}. Changes in size selectivity and functional response during life history were reported in insect predators of the genus *Notonecta*^{28,32,33}. Within the five larval stages of *Notonecta*, early instars usually prefer small crustaceans, whereas later instars forage on larger prey³⁴.

In this study, we investigated the interaction of predation and toxic stress using an integrative approach of laboratory experiments and independent mechanistic model simulations. In our laboratory experiments, we exposed *Daphnia* populations to pulses of a toxicant, to continuous predation by *Notonecta* alone, and to a combination of both. To capture size-selective effects, not only abundance but also size-structure was recorded. To explore scenarios and hypotheses beyond the constraints of our experiment, an individual-based model of our system was used to test effects of predator size, predation intensity and the significance of certain, isolated mechanisms on the *Daphnia* population's resilience.

Results

Experimental results. Population size. Size of *Daphnia magna* populations increased during the first four weeks of the experiment. After reaching maximum abundance, the total number of daphnids decreased and control populations leveled off after reaching environmental capacity at a mean density of 113.4 ± 31.49 (mean \pm standard deviation) individuals per L (Figure 1, details given in Appendix S3 in Supporting Information). The density of *D. magna* under predation did not significantly differ from control with a single exception 9 days after introduction of backswimmers (Figure 1, $P = 0.036$). Thereafter, between day 14 and day 34 of the experiment (hereafter referred to as the recovery phase), total abundances of *Daphnia* slightly increased compared to control, with differences not being significant. The first p-353 nonylphenol pulse exposure resulted in a significant short-term reduction in *D. magna* population size as compared to control ($P = 0.036$), whereas the second nonylphenol pulse did not have a significant effect on the total daphnid abundance ($P = 0.158$). In the systems exposed to both predator and nonylphenol, population density did not significantly differ from single stressors until day 40 of the experiment ($P = 0.065$), including the first pulse exposure. Following the second pulse exposure the total number of *Daphnia* was significantly reduced compared to predation and nonylphenol treatments ($P = 0.022$). Finally, one out of three *Daphnia* populations exposed to the combined stressor went extinct and the other two populations were dramatically reduced in size (Figure 1); only in one of the three replicates did the population abundance start to increase again on the last day of the experiment.

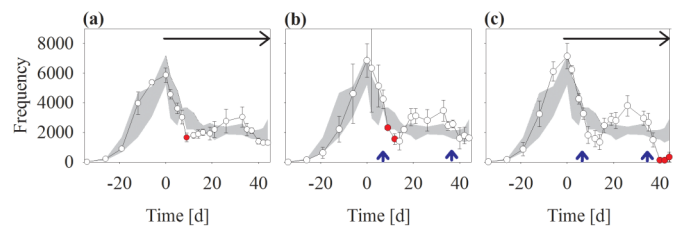


Figure 1 | Total number of *Daphnia magna* during the course of the experiment. *Daphnia* populations were exposed to three different disturbance scenarios: (a) *Notonecta maculata* predation, (b) pulse exposures of p353-nonylphenol, and (c) combined disturbance by means of nonylphenol and predation. The black arrow marks the period of predator exposure, blue arrows indicate nonylphenol two-day pulse exposure periods. Dots represent mean and standard deviations of three replicated measurements. The grey shade symbolizes the range of control populations (range between minimum and maximum control abundance) and red dots indicate significant difference from control in ANOVA with subsequent Tukey post-hoc test.

Population size-structure. Comparison between first (day 7) and second (day 35) nonylphenol pulse exposures revealed that size-structure of control populations had changed during the course of the experiment (Figure 2), in particular, the number of adults (> 2.6 mm) steadily increased with time. Although backswimmer predation did not greatly affect total abundances, we observed alterations in *D. magna* population size-structure. The comparison of size distribution between control and disturbed populations (Figure 2) suggests that small sized *D. magna* were predominantly reduced by the predation of 1st instar backswimmers. Abundance of juvenile daphnids (> 1.3 mm & ≤ 2.6 mm) was significantly lower in predation systems as compared to control nine days after backswimmer introduction ($P = 0.01$). By contrast, in the presence of older backswimmers, numbers of adult *D. magna* were significantly reduced at the end of the recovery phase (from day 33, $P < 0.001$, Figure 3). The lack of significant differences in total abundance between predation and control can thus be attributed to the significantly higher numbers of neonate *D. magna* from day 37.

In single stressor settings, *D. magna* neonates were most severely affected by nonylphenol treatments (Figure 2). During the first exposure period, neonates were reduced in numbers by a factor of 12.3 and a significant difference from control was apparent three days after termination of the nonylphenol treatment ($P = 0.002$). During the recovery phase, neonate abundance increased and significantly exceeded control level ($P = 0.014$). The second nonylphenol treatment reduced the number of neonate *D. magna* by a factor of 5.7 whereas the difference to control treatment was, however, not significant. Abundances of juvenile and adult daphnids were significantly reduced during the first nonylphenol exposure period by a factor of ~ 2 ($P = 0.01$, $P = 0.028$ respectively). Thereafter, the number of both juveniles and adults did not significantly differ from control, with the exception of the last day of the experiment where a significant reduction in adult abundance was apparent ($P < 0.001$).

In combined stressor settings, the number of neonate *D. magna* was reduced by a factor of 13.9 as a result of the first nonylphenol pulse exposure. The size distributions of *D. magna* populations indicate that the effect of the first nonylphenol treatment was more pronounced in combined stressor systems compared to single stressors (Figure 2). However, abundances of the three size classes did not significantly differ from single stressor scenarios ($P = 0.06$). At the initiation of the second nonylphenol pulse exposure populations exposed to combined stressors largely consisted of small bodied daphnid specimens whereas adult *Daphnia* accounted for only 4.1% of the population. In the following days, neonate and juvenile abundance was significantly reduced ($P < 0.001$ and $P = 0.037$).

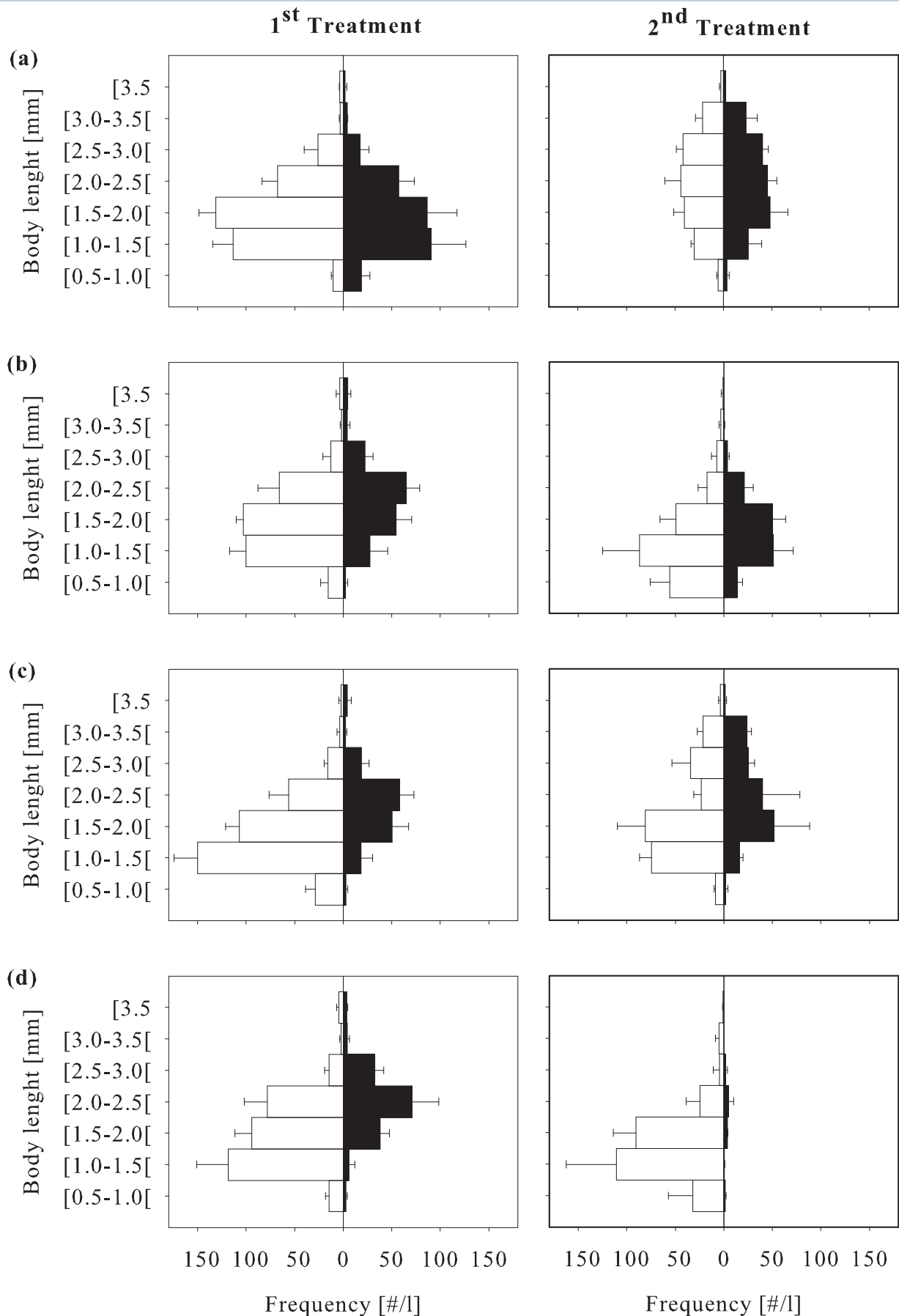


Figure 2 | Population demography of *Daphnia magna* based on body length measured before (white bars) and after (black bars) the first (left) and second (right) p353-nonylphenol pulse exposure period; bars represent mean and standard deviation of three replicates in a control (a) and three different treatments: (b) predation by *Notonecta maculata* juveniles, (c) exposure to nonylphenol, and (d) a combination of both nonylphenol exposure and predation.

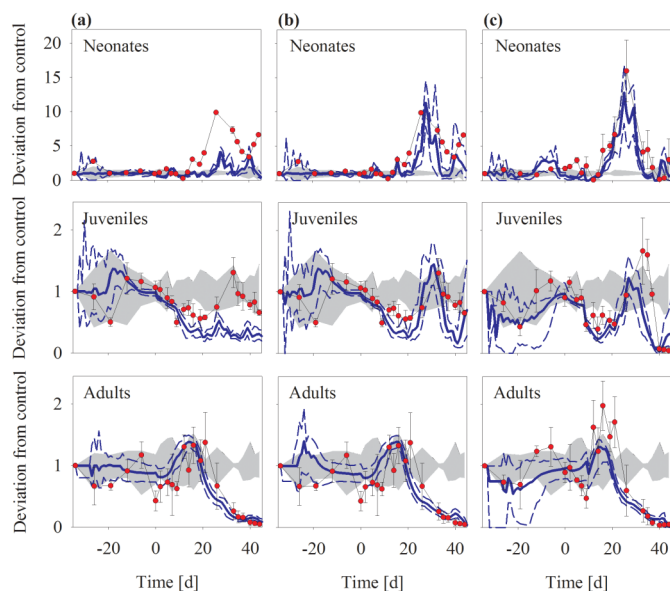


Figure 3 | Importance of life-history plasticity in *Daphnia magna* population response to predation (a and b) and both predation and chemical stress (c). Dynamics are shown separated by *D. magna* size classes within the populations: neonates (≤ 1.3 mm), juveniles (> 1.3 – 2.6 mm) and adult (> 2.6 mm). Simulated (blue lines) and measured population (red dots, mean \pm standard deviation, $n = 3$) dynamics are presented in relative deviation to mean control abundance, with measured control range (range between minimum and maximum control abundance relative to mean) represented by grey shades. Dotted lines indicate minimum and maximum and solid line represents mean of 100 Monte-Carlo simulations. Panel a shows simulated population response to *N. maculata* predation whereas in panels b and c additional effects of predator kairomones were simulated. Data points in a and b are identical.

respectively) by factors of 36.6 and 27.1 respectively as compared to pre-exposure conditions. In the end, adult *D. magna* were totally absent from two of the three combined stressor systems.

Toxicant concentrations and food conditions. Measured nonylphenol concentrations were on average 23.1% below the nominal concentration of $0.45 \mu\text{M}$ considered for the experiment (Table 1). Density of algae that were not consumed decreased during the time course and reached a plateau on day -6 (Table 2). No significant difference was found in the density of algae between control and treatment systems (Table 2), with the exception of day -12 ($P = 0.009$) indicating that populations equally reached carrying capacity of the systems following the peak density.

Modeling results. The individual-based population model was run with and without consideration of predator kairomone effects on *Daphnia* life history. Without kairomone effects the impact of the predator was due to feeding only whereas in kairomone simulations *D. magna* adults produced more but smaller neonates. The importance of life-history plasticity in *D. magna* becomes apparent when comparing neonate and juvenile dynamics predicted by the

two models (Figures 3A and 3B). Without consideration of kairomone effects, the model predicts only a slight increase in neonate abundance during the recovery phase of the experiments between days 15 and 30, largely as a consequence of increasing food availability. However, in order to capture the full magnitude of reproductive output and the subsequent increase in juvenile abundance, life-history plasticity needed to be included in the model. Consequently, with consideration of kairomone effects, the model is able to capture the dynamics of population size-structure reasonably well.

To examine the effect of predator size, predation intensity and the presence of kairomones on *Daphnia* population size-structure and on population vulnerability to the toxicant, we simulated larger environments that were able to carry more than a single backswimmer. Simulated extinction probabilities revealed that vulnerability of *D. magna* populations to chemical stress increases with predator size and predation intensity whereas the presence of kairomones buffered chemical effects to a certain extent (Figure 4). The patterns in the *D. magna* body size ratio (≤ 2.6 : > 2.6 mm) as function of nonylphenol concentrations differed remarkably between the two predator sizes tested (Figure 4). As a general trend, size ratios decreased with increasing chemical concentration, i.e. a shift towards older and thus larger animals. Simulated kairomone effects generally led to a shift towards small *D. magna* as a consequence of higher reproductive outputs. In small predator simulations, the stepwise reduction of the size ratio with increasing predation intensity can be ascribed to the increasing predation pressure on small *D. magna*. For large predators, the preference for large daphnid prey led to more complex size ratio patterns: under low nonylphenol exposure concentrations, size ratio increased with increasing predation pressure because small *Daphnia* could emerge and grow due to reduced intraspecific competition for algae. In contrast, under high exposure a large fraction of small *Daphnia* was killed leading to an inverse size ratio trend. These effects were even more pronounced under kairomone effects. Higher predation pressure of the large *N. maculata* instar led to rapid changes in size-structure and extinction probability, irrespective of life-history plasticity in *D. magna*. The modelling results support the experimental findings that selective predation of large backswimmer instars promoted size classes that were more vulnerable to the toxicant leading to higher vulnerability in populations exposed to a combination of both chemical and natural stressor.

Discussion

Population dynamics of the cladoceran filter feeder *Daphnia magna* were observed and simulated for different disturbance scenarios including pulse exposure to p353-nonylphenol, predation of the insect predator *Notonecta maculata*, and a combination of both. Effects of the single stressors on total abundance were almost entirely cryptic, i.e. hardly detectable. In contrast, the combination of predation by large backswimmers and the second nonylphenol pulse exposure drove the daphnid populations to the brink of extinction (Figure 1). In the following section we will provide a mechanistic explanation of the interactive effects observed. Furthermore, we will discuss what we have learned about buffer mechanisms and cryptic effects, followed by a discussion of the relevance of our findings for ecological risk assessments of chemicals.

Table 1 | Mean and standard deviation of measured nonylphenol concentration in test media ($n = 3$) during the first (days 7 and 8) and second (days 35 and 36) 2-day pulse exposure period. NP: p353-nonylphenol exposure; Np-Nm: combined stressor

Treatment	p353-nonylphenol concentrations [μM]			
	Day 7	Day 8	Day 35	Day 36
NP	0.47 ± 0.163	0.24 ± 0.088	0.38 ± 0.192	0.16 ± 0.070
NP-Nm	0.37 ± 0.122	0.18 ± 0.028	0.18 ± 0.136	0.10 ± 0.26



Table 2 | Mean and standard deviation of food availability ($n = 3$) given as total organic carbon [mg/L], letters indicate differences in one way Anova with subsequent Tukey HSD post-hoc test. C: control, Nm: *Notonecta maculata* predation, NP: p353-nonylphenol exposure; Np-Nm: combined stressor

Day	C	Nm	NP	NP-Nm	p
-26	4 ± 0.29^A	4.6 ± 0.98^A	4.3 ± 1.36^A	4.7 ± 1.55^A	0.120
-19	3.8 ± 1.55^A	6.6 ± 1.08^A	5.6 ± 0.42^A	6.1 ± 3.63^A	0.956
-12	1.3 ± 0.2^A	2.6 ± 0.89^B	3.3 ± 1.07^B	4 ± 0.38^B	0.009
-6	1.2 ± 0.36^A	1 ± 0.15^A	1.7 ± 0.06^A	1.3 ± 0.23^A	0.105
5	1.3 ± 0.71^A	1.4 ± 0.64^A	1.7 ± 0.26^A	2.1 ± 0.11^A	0.288
16	1 ± 0.17^A	1.5 ± 0.23^A	1.1 ± 0.21^A	1.2 ± 0.23^A	0.221
26	1.7 ± 0.11^A	1.2 ± 0.41^A	1.6 ± 0.15^A	1.2 ± 0.25^A	0.135
36	1.4 ± 0.47^A	1.4 ± 0.26^A	1.4 ± 0.64^A	1.5 ± 0.52^A	0.476

Total abundance of *Daphnia magna* was only marginally affected by the predation of backswimmers. Similarly, laboratory populations of *Daphnia pulex* were able to persist when exposed to groups of *Notonecta hoffmani* with abundances not being significantly different to control systems³⁵. The authors suggested that an increase of reproductive output and juvenile survival probably due to a release from starvation enables prey populations to maintain densities and compensate for predation. Obviously, the loss of individuals due to predation was buffered by changes in the internal organization and size-structure of *Daphnia* populations.

Using a realistic and mechanistic population model, we have shown that altered food conditions in combination with effects of life-history plasticity due to predator kairomones, led to increased reproduction and smaller offspring size (Figure 3). Only this combination of responses to predator effects could explain why effects on population dynamics, i.e. abundance, were cryptic while population size-structure changed dramatically.

Similar to fish, *Notonecta* species are usually considered as gape-limited predators preferring larger prey²⁰. However, small backswimmer instars prefer small over larger prey²⁸. Accordingly, early in the

experiments differences in population size-structure in control populations can partly be ascribed to size-selective predation in *N. maculata* juveniles³². This resulted in a reduction of neonate and juvenile *D. magna*, although the impact of predation was limited due to lower feeding rates in small instars³⁶. Later in our experiment, larger backswimmers with more intense predation and a preference for larger prey reduced the abundance of adult *D. magna* to extinction and thereby induced a significant shift in the size-structure of populations towards smaller daphnids.

Our model revealed that kairomone effects were important to fully capture the observed response of the *Daphnia* population to predators (Figure 3). When exposed to *Notonecta* kairomones daphnids tend to produce larger clutch sizes with individual neonates being smaller, thereby influencing offspring vulnerability to the chemical stressor. These effects are an example of non-trophic, trait-mediated interactions. Their relevance for community dynamics and structure is increasingly noticed³⁷, a prominent example being ungulates in the Yellowstone National Park who forage in vegetation with better cover but less nutritional quality since wolves have been re-introduced³⁸. Our study demonstrates that such effects also have to be taken into

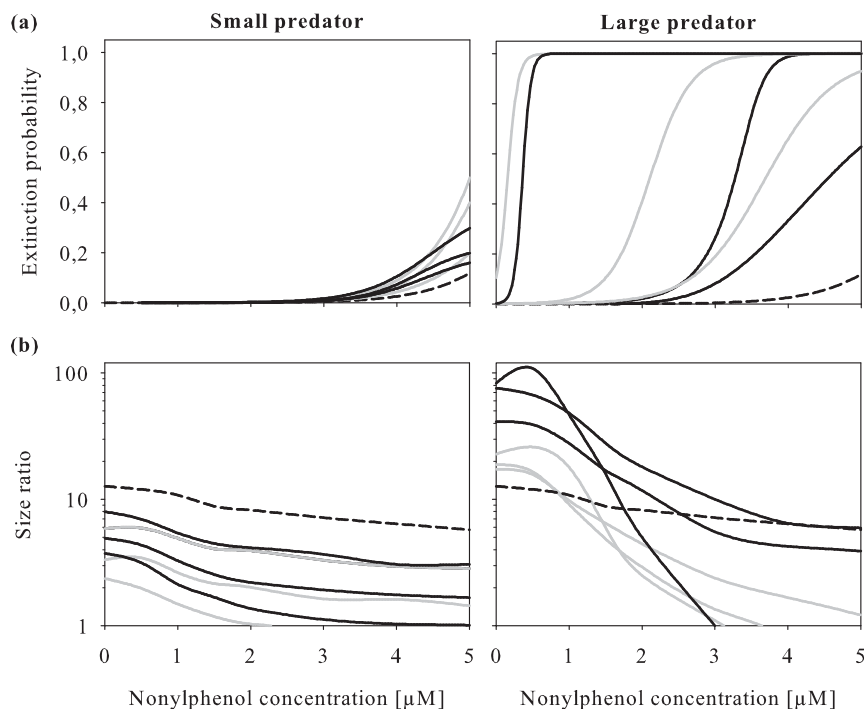


Figure 4 | Extinction probability (a) and size ratio (b) in *Daphnia magna* populations as functions of nonylphenol concentration simulated without (grey solid lines) and with (black solid lines) consideration of kairomone effects. Endpoints were calculated for small (1st instar, left panel) and large (5th instar, right panel) *Notonecta maculata*. Increasing line weight corresponds to increasing predation intensities (1–3 *N. maculata* specimens per system), with dashed lines representing simulations without predation. Extinction probabilities were recorded 10 days after initializing a 2-day pulse exposure and effects on population size-structure (mean of 100 Monte-Carlo simulations) 3 days after initializing exposure.



account if the ecological realism of risk assessment of chemicals is to be improved.

Another important mechanism causing alterations in the size-structure of *D. magna* populations in response to stress was intra-specific competition. A large number of neonate *D. magna* were produced when total abundance was below the carrying capacity of the experimental systems, i.e. during the initial phase of the experiment and after disturbances. As soon as the carrying capacity was reached, intra-specific competition for food and space presumably caused a reduction in reproduction, growth and survival. Reductions in the reproductive output and survival at individual level have previously been reported for *D. magna* as a consequence of food shortage^{39–41} and crowding^{41,42}.

The toxicant used in our experiments, p353-nonylphenol, has previously been found to act in a size-dependent manner, with juvenile and adult *D. magna* being less sensitive than neonates⁴³. Consequently, in accordance with the study of Agatz and co-workers⁴⁴, we found that populations of predominantly small daphnids have been affected by lower nonylphenol pulse exposure. However, in our study, the response of the *Daphnia* populations to toxic stress differed with timing of exposure. A significant effect was observed at the first peak, whereas the second peak showed no significant effect on abundance. During the initial phase of the experiment small *Daphnia* were numerically dominant; this is typical for growing populations⁴⁵. Therefore, the first nonylphenol pulse exposure affected a significant proportion of the population. Then, in the non-predated systems the proportion of larger *D. magna* steadily increased over time. Accordingly, the proportion of small-sized *Daphnia* that might be mostly affected by the toxicant was lower during the second exposure period and thus toxic stress did not result in a significant reduction in population size. With predation on the other hand, first small backswimmers reduced the number of small daphnids so that only a small proportion of the *Daphnia* populations were affected by the toxicant at the first peak. Consequently, the first nonylphenol exposure did not result in a significant difference between the predated systems and the ones exposed to the combined stressor.

Later in the experiment, predation of large backswimmers foraging on larger daphnids and the high reproductive output induced by reduced intraspecific competition led to a shift in population size-structure towards small *Daphnia* at the time of the second toxicant exposure. With small daphnids being more sensitive, a large proportion of the population was affected by the toxicant and finally became extinct or was driven to the brink of extinction.

In contrast, both intraspecific competition for food, leading to the well-known cohort-dominance suppression cycles⁴⁶, and episodic addition of food are unlikely to explain our observations per se, as they are represented in the simulation model. However, spatial effects such as swarming and vertical migration might have effects. We observed that migration to the bottom of the aquarium was less pronounced in the absence of predators. Since our simulation model is non-spatial, it does not account for such effects, which might explain why population dynamics and size-structure predicted by the model are, although capturing the main trends and levels, not 100% perfect.

The above findings have implications for the analysis of resilience and multiple stressor scenarios. In ecology, resilience has become a central concept. It includes the notion of buffering disturbances to a certain limited capacity which can be exhausted by too strong or too long-lasting a stress. The reason for the limited capacity is that stressors have to cause some change in the system, otherwise they would not be considered stressors. In ecotoxicology, multiple stressors are customarily discussed in close connection with synergism. Synergistic effects are claimed whenever a combination of stressors amplifies effects, usually observed on the level of the individual organism, beyond additivity⁴⁷. In our study, the combination of a natural and chemical stressor did not increase the effects on the organismal level

but led to a nonlinear combination of effects at the population level. The effect of predation on prey individuals was buffered by reproduction at population level, subsequently leading to a shift in population size-structure (Figures 2 and 3). The effect of a second stressor finally led to the loss of resilience.

If resilience is lost then abrupt regime shifts occur, leading to a system that is organized in a fundamentally different way⁴⁸. Our study is one of the first which, for a population under multiple stresses, combines experiments and modeling to provide mechanistic explanation of resilience and the loss of its buffering capacity. The lesson learned from our study is that in general the response of a population or ecological system to single stressors inevitably causes changes in structure and internal organization. These responses have different profiles, each changing the susceptibility to other stressors in a particular way. Assessing risk of single stressors might thus severely underestimate real risk. Ecologically relevant risk assessment requires a more comprehensive view including population structure and internal organization.

Traditionally, population ecology has a strong focus on abundance and it can be difficult enough to estimate abundance in real populations. However, in theoretical ecology, structured models⁴⁹ and individual-based models⁵⁰ are increasingly used to take into account differences among individuals and their adaptive behavior, including plasticity in physiology and life history. Population size is a summary statistic that ignores information about population structure and the state and behavior of individuals that in many, if not most, cases is vital to understand and predict the response of populations to changing conditions, including stress.

Nevertheless, population level endpoints (i.e., metrics for risk assessment) currently discussed in ecological risk assessment of chemicals still focus on abundance itself or abundance-related metrics: population growth rate⁵¹ or recovery of abundance to pre-disturbance levels⁵². These endpoints certainly contain important information, but our study has demonstrated that endpoints based on abundance might not detect real risk if, as in the current practice in risk assessment of chemicals, only single stressors and single populations are considered. As demonstrated in our study, effects of a toxicant can go undetected when ignoring population structure but become relevant when combining chemical stress with species interactions, both direct and trait-mediated.

For populations, demographic studies that include estimates of population vulnerability resulting from size-dependent differences in susceptibilities to toxicants offer a starting point in overcoming the limitation of traditional toxicity assessments based on individual organisms⁵³. For communities, a multitude of factors and interactions such as competition, predation or parasitism complicate the empirical assessment of toxicant effects. Even if one or more of these interactions are addressed, results may depend on experimental settings, as shown here for the case of predation (Figure 4). The strong interaction observed in our experimental system appears to be largely due to strong size-dependent effects, in particular due to the relative sizes of predators and prey. In a natural system one might expect a more heterogeneous distribution of predator sizes and size-selectivity which need further investigation. We thus conclude that mechanistic and well-tested population and community models are needed to fully understand mechanisms behind the interaction of natural and chemical stressors. Routinely using experiments and models together, as in the present study, will ultimately help to develop schemes for a more realistic ecological assessment of chemicals.

Methods

Experimental study. In a laboratory experiment, we observed *Daphnia magna* population dynamics under pulsed stress of 4-(3,5 dimethyl-3 heptyl)phenol (p-353 nonylphenol), under predation by the backswimmer *Notonecta maculata* and with a combination of both, toxic stress and predation.



Test Animals. The experiment was carried out using *D. magna* clone 5. Daphnids were reared as described by Siehoff and colleagues⁵⁴. Adults of *N. maculata* were originally collected from an outdoor mesocosm site and were cultured under laboratory conditions. Backswimmer juveniles were reared individually and fed *ad libitum* with daphnids. For culturing and experiments, animals were kept in a constant room set at $20 \pm 1^\circ\text{C}$ and a 16 h : 8 h (light:dark) photoperiod. Artificial freshwater M4 was used as a medium⁵⁵.

Experimental setup. The *D. magna* population experiment was conducted under semi-static conditions using 20 L glass aquaria. Replicate populations ($n = 3$) were initiated on day -34 with four 21–28 day old *D. magna* specimens as well as 10 third brood neonates that were < 24 h old. The development of populations was followed during a period of 78 days. Daphnids were fed with the green algae *D. subspicatus* in a concentration of 6 mg TOC day⁻¹ population⁻¹ until day -25, 15 mg between day -24 and day -11 and 12 mg from day -10. Food was provided daily on working days with the amount of algae tripled on Fridays. In all aquaria dead daphnids, carcasses, aborted or ephippial eggs and deposited algae were removed weekly using a suction hose and half of the medium was renewed.

N. maculata specimens (< 24 h old) were placed in prepared aquaria at *Daphnia* peak abundance on day 0 of the experiment; they grew from first to fifth instar during the remaining period of the test. Backswimmers were checked daily and dead ones were replaced by individuals of the same age. Two two-day nonylphenol pulse exposures were initiated on day 7 and day 35 respectively. For nonylphenol treatment a nominal concentration of 0.45 μM was chosen, which was below the EC_{50} value in *D. magna* acute 48 h-toxicity test using neonates⁴³.

Sampling procedure. Control and treatment populations of *D. magna* were sampled weekly at the beginning of the experiments and up to three times per week after the introduction of backswimmers. For the quantification of *D. magna* population size and size-structure, a sample of 2 L was obtained from each aquarium by taking 28–30 subsamples in a predefined pattern. For sampling, a plastic tube (30×2 cm) was vertically plunged into the water column. The upper end of the tube was sealed, but a small opening allowed air to leave the cylinder. Removal of enclosed *Daphnia* and water from the aquaria was possible when closing the upper opening. *Daphnia* samples were sieved (300 μm mesh), transferred to petri dishes and were returned to the aquaria after scanning. Daphnids were counted and body lengths of individuals (excluding spine) were measured digitally.

Density of algae cells was measured photometrically (Hitachi 100–40, 720 nm) on eight occasions during the course of the experiment. Therefore, a 50 ml sample of test medium was taken from each aquarium prior to feeding with algae. Total organic carbon was estimated from the empirical regression $\text{TOC} = 269.73 \text{ A} / (2.76 - \text{A})$ with A being the measured absorbance.

Nonylphenol exposure. P-353 nonylphenol was synthesized as described previously⁵⁶, yielding a chemical purity of $> 97\%$. Acetone (Roth, purity 99.9%, Rotisolv-HPLC) was used as a carrier solvent. Aliquots of the nonylphenol stock solution (27.3 μM) were added to 200 ml medium and stirred before treating full aquaria. Nonylphenol exposures were terminated by fully replacing media after carefully cleaning aquaria with ethanol and water. Controls and predation systems were treated in the same way. Exposure concentrations were verified as described in Appendix S1 in Supporting Information.

Data analysis. For statistical analysis daphnids were graded into three size classes; these were neonates (≤ 1.3 mm), juveniles (> 1.3 and ≤ 2.6 mm) and adults (> 2.6 mm). Differences in *D. magna* total abundance and abundances within daphnid size classes between control and disturbance scenarios were tested by means of a one-way analysis of variance (Anova) with subsequent Tukey-HSD posthoc analysis, since most data passed Levene's test of homoscedasticity and Shapiro-Wilk test of normal distribution. Statistical analyses were conducted using PASW Statistics 18 (SPSS Inc., 2009).

Modeling study. In order to explore scenarios and hypotheses beyond the constraints of our experiment, we applied an individual-based population model of our predator-prey-toxicant system. Submodels were developed, parameterized and tested independently of the current study^{28,33,41,57}. For modeling details see Appendix S2 in Supporting Information.

We ran the model for two different scenarios, (I) in accordance with the settings of our laboratory experiment as described above, applying nominal chemical concentrations and (II) in larger environments that were able to carry more than a single backswimmer under control conditions in order to examine the effect of predator size, predation intensity and the presence of kairomones on *Daphnia* population dynamics. For the latter scenario, simulation settings differed from the experiments as follows. We used 150 l environments and supplied *Daphnia* populations with food at a rate of 25 mgC d⁻¹. Notonectids were placed into the systems on simulation day 40, and 2-day nonylphenol pulse exposures were initiated on day 60. For simulations we used the predator profiles of the 1st and 5th instar backswimmers and assumed size to be constant. We calculated the ratio of small daphnids (neonates + juveniles) and adults as a measure for population size-structure on simulation day 63 and the extinction probability, i.e. the fraction of populations that went extinct in 100 Monte-Carlo simulations, on simulation day 70.

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Author contributions

A.G. designed and carried out the experiment, performed modeling work and analyzed data, A.Z. analyzed chemical concentrations, T.G.P. designed the experiment and performed modeling work, A.G. and V.G. wrote the first draft of the manuscript and all authors contributed substantially to revisions.

Additional information

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